Rank	Scores Model	Local	Sequence	(Position)
F1	6	1	GGTAACGGCTCACCTAGGC	(246)
F2	7	1	CCAGATGGGATTAGCTAGTAGG	(221)
F3	8	1	GACCAGGGCTACACACGTG	(1196)
F4	8	1	GTAACGGCTCACCTAGGCG	(247)
F5	9	1	GGGTAACGGCTCACCTAGG	(245)
F6	10	1	TAACGGCTCACCTAGGCGA	(248)
F7	10	1	CCCAGATGGGATTAGCTAGT	(220)
F8	11	1	AAACGATGTCGACTTGGAG	(797)
F9	11	1	AGGCGACGATCCCTAGCTG	(261)
F10	11	1	TAAACGATGTCGACTTGGAG	(796)
F11	12	1	GAAGTCGGAATCGCTAGTAAT	(1312)
F12	13	1	AAGACCAAAGAGGGGGACC	(176)

Rank	Scores Model	Local	Sequence	(Position)
R1	5	1	CCGTTACCCCACCTACTAGC	(253)
R2	6	1	TAGGGATCGTCGCCTAGGT	(275)
R3	7	1	GCCGTTACCCCACCTACTAG	(254)
R4	8	1	CTAGGGATCGTCGCCTAGG	(276)
R5	8	1	CGCCATTGTAGCACGTGTG	(1225)
R6	9	1	AGGGATCGTCGCCTAGGTG	(274)
R7	9	1	CGTTACCCCACCTACTAGCT	(252)
R8	10	1	CGTTACCCCACCTACTAGCTA	(252)
R9	11	1	CTGATCCACGATTACTAGCG	(1342)
R10	11	1	TCCTCTCAGACCAGCTAGG	(290)
R11	11	1	AAGGGCACAACCTCCAAGT	(826)
R12	12	1	TCATCCTCTCAGACCAGCTAG	(293)

Figure 21. Primer Selection Search Results

III Hybridization Temperature Calculation

Hybridization is usually few degrees lower than the melting temperature. There are many published models to calculate the melting temperature. Most of them are based on the following simplified equation:

$$Tm = 81.5 + 16.6*\log_{10}(C_{Na}) + 41*f_{GC} - 600/n_{total}$$